REMARKS

The Office Action

Claims 1-19, and 58 are pending. Claims 1-13, 15-17, and 18-19 stand rejected for lack of written description. Claims 1-19, and 58 stand rejected for lack of enablement.

Amendments to the Specification

The specification has been amended to include the status of the priority documents and to include an abstract of the disclosure. References to hyperlinks have also been removed.

Rejections under 35 U.S.C. 112, first paragraph

Claims 1-19 are rejected under 35 U.S.C. 112, first paragraph, for written description and/or lack of enablement. The Examiner states:

> [T]here does appear to be allowable subject matter, if for example, if claim 1 incorporated the limitations of claim 9 and claims 14-15 because the prior art does not appear to teach or suggest a method for stimulating an immune response specific toward a naturally occurring protein comprising administering an altered protein or polypeptide fragment thereof derived from said naturally occurring protein, wherein an unstable polypeptide segment has been inserted by artifice into said altered protein, wherein immunogenicity of the naturally occurring protein is increased and wherein said segment has the properties recited in instant claims 14 or18.

The Examiner has indicated that claims 1-19 would be fully enabled and not subject to written description rejection, if the limitations of claim 9 and 14 are incorporated into claim 1. Applicant now amends claim 1 to include the limitations of claims 9 and 14, now canceled. Amended claim 1 now recites the definition of "unstable polypeptide segment" given in the specification and the limitation that the immunogenicity of the naturally-occurring protein be increased. Since claim 1 has been amended according to the Examiner's suggestion, the rejection of claims 1-19 may be withdrawn.

Claim 58 is rejected under 35 U.S.C. 112, first paragraph for lack of enablement. Amended claim 58 recites:

> A method for stimulating an immune response toward naturally-occurring HIV gp120 protein in a human, said method comprising administering to said human an altered HIV gp120 protein, wherein a human Hsp10 mobile loop has been inserted by artifice into said altered HIV gp120 protein.

The Examiner states, "[I]t is not clear how increasing the immunogenicity of the gp120 protein by said modification ... stimulates an immune response toward <u>any</u> naturally occurring protein other than gp120." (emphasis in original) As now amended, claim 58 is limited to stimulating an immune response toward HIV gp120. The Examiner further asserts, "There is insufficient guidance and direction that said insertions even stimulate an immune response to any protein, including gp120..." Applicant respectfully disagrees. The mobile loop of Hsp10 is a specific example of an unstable polypeptide segment as defined by the specification. The specification provides ample teaching on how the insertion of such unstable polypeptide segments into a naturally occurring

protein increases the immunogenicity of that protein. In addition, pp. 40-56 of the specification describe, in detail, the method of claim 58. In sum, claim 58, as amended, is enabled, and the rejection should be withdrawn.

CONCLUSIONS

Applicant submits that the claims are now in condition for allowance, and such action is respectfully requested. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Version with markings to show changes

In the Specification

Fig. 11A is a graph showing the correlation of T-cell epitopes with sequence conservation in HIV gp120. Epitope identifications were obtained from the HIV Immunology Database, Los Alamos National Labs [(http://hiv-web.lanl.gov)], which includes a compilation of epitopes published prior to the year 1995 (see Fig. 11B). Sequence positions found in multiple epitopes are scored multiple times. Epitope scores were smoothed by an 11-residue averaging window. Sequence conservation was evaluated for twenty-three gp120 sequences. The most popular residue at each position was determined, and then scored for its use at that position in each sequence. Conservative substitutions were counted the same as identical matches. Conservation scores were averaged with a 11-residue window. Epitopes are located in regions with above average conservation (>0.87).

Fig. 11B is the sequence of HIV gp120 (SEQ ID NO: 1) obtained from the HIV Immunology Database, Los Alamos National Labs [(http://hiv-web.lanl.gov)] showing the 36 epitope identifications of epitopes published prior to the year 1995.

Figs. 13A, 13B, and 13C are graphs showing the combination of predictive methods based on MHC binding and preferred processing at poorly conserved regions. In Fig. 13A, the raw experimental epitope scores are shown. In Fig. 13B epitopes were predicted by the EpiMatrix analysis [available at http://hiv-web.lanl.gov/immuno/articles/LANL.html] (no geographic bias in MHC preferences, 20% match to motifs allowed). In Fig. 13C, the EpiMatrix scores were set to zero when the sequence conservation at that residue fell below the average for the whole protein. Note in particular the elimination of predicted epitopes in the segment 130-200.

As was observed for lysozyme, *M. leprae* cpn10, and staphylococcal nuclease, helper T-cell epitopes in *H*uman *I*mmunodeficiency *V*irus (HIV) gp120 tend to cluster near sites that may be preferentially cleaved during antigen processing (Fig. 6). Epitopes were defined using a variety of T-cell stimulation systems, for example, with lymphocytes from draining lymph nodes of mice immunized with native gp120 (Cease *et al.*, Proc. Natl. Acad. Sci. USA 84: 4249-4253, 1987), peripheral blood lymphocytes from humans immunized with vaccinia virus expressing gp120 (Berzofsky *et al.*, Nature 334: 706-708, 1988), and peripheral blood lymphocytes from HIV patients (Clerici *et al.*, Nature 339: 383-385, 1989). Data have been collected and published on the World Wide Web in the HIV Immunology Database, Los Alamos National Labs [(http://hiv-web.lanl.gov)]. Epitopes are broadly distributed over the C-terminal half of gp120. Fewer epitopes occur in the N-terminal half of the protein, although there is a cluster in the region, 101-119, including the "T2" epitope (Cease *et al.*, *supra*). Overlapping epitopes may be grouped into eight regions (shown as gray boxes in Fig. 6) that encompass most of the gp120 sequence: 31-54, 64-84, 101-119, 203-269, 273-301, 306-369, 417-453, and 457-502. However, several much shorter segments are over-represented in the sample of reported epitopes. We define immunodominant sequences as those occurring in at least four epitopes: 104-115, 225-236, 294-297, 311-349, 426-440, and 486-500. The number of immunodominant regions (shown as black boxes in Fig. 6) is very similar to the number of solvent-exposed segments (shown as white boxes in Fig. 6) identified by monoclonal antibodies that bind to linear epitopes in native gp120 (Moore *et al.*, J. Virol. 68: 469-484, 1994), and the immunodominant regions tend to be adjacent to the solvent-exposed segments. Three of these solvent-exposed segments also were preferentially susceptible to proteolysis. Taken together, these observations demonstrated that proteolytic nicking targets presentation of nearby sequences in HIV gp120.

In the Claims

1. (Amended) A method for stimulating an immune response specific toward a naturally-occurring protein in an animal having an immune system including T cells, said method comprising administering to said animal an altered protein or polypeptide fragment thereof derived from said naturally-occurring protein, wherein an unstable polypeptide segment has been inserted by artifice into said altered protein, wherein said unstable polypeptide segment has an average hydrophobicity value that is lower than the average hydrophobicity value of said altered protein; has a sequence conservation that is lower than a sequence conservation of said altered protein; has an amide protection factor that is lower than 10^4 wherein said altered protein is in a native conformational state; has an average amide protection factor that is lower than the average amide protection factor for said altered protein in a denatured conformational state; has an NMR order parameter (S^2) of less than 0.8; or has an average B-factor value that is higher than the average B-factor value of said altered protein, and wherein immunogenicity of said naturally-occurring protein is increased.

10. (Amended) The method of claim 1 [9], wherein said altered protein or polypeptide fragment thereof is in a vaccine.

11. (Amended) The method of claim 1 [or 9], wherein said unstable polypeptide segment comprises at least twelve amino acid residues.

13. (Amended) The method of claim 1 [or 9], wherein said unstable polypeptide segment comprises a polypeptide sequence that is specifically recognized by a protease.

15. (Amended) The method of claim 1 [or 9], wherein said altered protein comprises a T cell epitope.

58. (Amended) A method for stimulating an immune response toward naturally-occurring HIV gp120 protein in a human, said method comprising administering to said human an altered HIV gp120 protein, wherein a human Hsp[ ]10 mobile loop [said] has been inserted by artifice into said altered HIV gp[ ]120 protein.

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1. (Amended) A method for stimulating an immune response specific toward a naturally-occurring protein in an animal having an immune system including T cells, said method comprising administering to said animal an altered protein or polypeptide fragment thereof derived from said naturally-occurring protein, wherein an unstable polypeptide segment has been inserted by artifice into said altered protein, wherein said unstable polypeptide segment has an average hydrophobicity value that is lower than the average hydrophobicity value of said altered protein; has a sequence conservation that is lower than a sequence conservation of said altered protein; has an amide protection factor that is lower than 10^4 wherein said altered protein is in a native conformational state; has an average amide protection factor that is lower than the average amide protection factor for said altered protein in a denatured conformational state; has an NMR order parameter (S^2) of less than 0.8; or has an average B-factor value that is higher than the average B-factor value of said altered protein, and wherein immunogenicity of said naturally-occurring protein is increased.

2. The method of claim 1, wherein said naturally-occurring protein is from a pathogen.

3. The method of claim 2, wherein said altered protein or polypeptide fragment thereof is administered to said animal to prevent infection of said animal with said pathogen.

4. The method of claim 1, wherein said naturally-occurring protein is from a neoplastic cell,

5. The method of claim 4, wherein said altered protein or polypeptide fragment thereof is administered to said animal to inhibit growth of said neoplastic cell in said animal.

6. The method of claim 1, wherein said altered protein or polypeptide fragment thereof is administered with a pharmaceutically acceptable carrier, an adjuvant or both.

7. The method of claim 1, wherein said animal is a mammal.

8. The method of claim 7, wherein said mammal is a human.

10. (Amended) The method of claim 1, wherein said altered protein or polypeptide fragment thereof is in a vaccine.

11. (Amended) The method of claim 1, wherein said unstable polypeptide segment comprises at least twelve amino acid residues.

12. The method of claim 11, wherein not more than 30% of said amino acid residues are selected from the group of amino acid residues consisting of isoleucine, leucine, valine, tyrosine, phenylalanine, tryptophan, threonine, and methionine.

13. (Amended) The method of claim 1, wherein said unstable polypeptide segment comprises a polypeptide sequence that is specifically recognized by a protease.

15. (Amended) The method of claim 1, wherein said altered protein comprises a T cell epitope.

16. The method of claim 15, wherein said unstable polypeptide segment is inserted N-terminally adjacent to said T cell epitope.

17. The method of claim 15, wherein the C - terminal portion of said unstable polypeptide segment overlaps the N - terminal portion of said T cell epitope.

18. The method of claim 15, wherein said T cell epitope has an average hydrophobicity value that is higher than the average hydrophobicity value of said altered protein; has a sequence conservation that is higher than a sequence conservation of said altered protein; has an amide protection factor that is greater than 10^4 wherein said altered protein is in a native conformational state; has an average amide protection factor that is higher than the average amide protection factor for said altered protein in a denatured conformational state; has an NMR order parameter (S^2) of greater than 0.7; or has an average B-factor value that is lower than the average B-factor value of said altered protein.

19. The method of claim 15, wherein at least 30% of the amino acid residues of said T cell epitope are selected from the group of amino acid residues consisting of isoleucine, leucine, valine, tyrosine, phenylalanine, tryptophan, threonine, and methionine.

58. (Amended) A method for stimulating an immune response toward naturally-occurring HIV gp120 protein in a human, said method comprising administering to said human an altered HIV gp120 protein, wherein a human Hsp 10 mobile loop has been inserted by artifice into said altered HIV gp120 protein.